Application of alizarin red S as an ion-pair reagent for the spectrophotometric determination of Olopatadine Hydrochloride in pharmaceutical formulation

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Abstract. A simple, accurate and precise spectrophotometric method has been developed and validated for the determination of olopatadine hydrochloride in bulk and their pharmaceutical formulation. This method was based on the formation of (yellow) ion – association complex with Alizarin Red S as chromogenic reagent in acidic medium, which is extracted into chloroform. The complex has a maximum absorbance at 425 nm. All variables affecting the reaction were studied and optimized. Beer's law was obeyed in the concentration range of 10–60 µg/mL. The validity of the described method was assessed. The method was successfully applied to the determination of olopatadine hydrochloride in its pharmaceutical formulation.

Keywords: olopatadine hydrochloride, alizarin red S, ion-pair reagent, spectrophotometric, pharmaceutical formulation

1. INTRODUCTION

Olopatadine Hydrochloride is an anti-histaminic with selective H1-receptor antagonist activity . Chemically it is (Olopatadine ,11-[(Z)-3-(dimethylamino) propylidene]-6,11-dihydrodibenz [b,e] oxepin-2-acetic acid mono hydrochloride) (Ohmori et al., 2004; Ohmori et al.,2002) . It is commonly used for the treatment of allergic rhinitis, eczema dermatitis ,chronic urticaria ,pruritis cutaneous, psoriasis vulgaris , erythema exsudativum multiform and treatment of allergic conjunctivitis. Olopatadine Hydrochloride is commercially available as tablets, ophthalmic solution and nasal spray. The determination of olopatadine hydrochloride is not yet described in any pharmacopoeias. Therefore, a simple, accurate method is required for their determination in pharmaceutical formulations.

Few methods have been described for the quantitative determination of olopatadine hydrochloride included spectrophotometric (Dey et al., 2010; Annapurna, et al., 2012) , HPLC-MS (Fujita et al.,1999; Zhu et al.,1999;Weili et al., 2009; Wei et al., 2006; Fujimaki et al., 2006) , HPLC (Wen-Fang et al., 2010; Wei-Sheng, Chang-Qun., 2007; Rao et al., 2012) and HPTLC (Anand et al.,2010).

Spectrophotometry is probably the most convenient analytical technique for routine analysis because of its inherent simplicity, low cost and wide availability in quality control laboratories. However, some of spectrophotometric methods reported for determination of olopatadine hydrochloride were associated with some major drawbacks, because of maximum absorption peak (λ_{max}) at 206 nm (Dey et al., 2010). Because of the highly blue shifted of above λ_{max}, their determination in the dosage forms based on the direct measurement of their absorption in UV spectral range is susceptible to potential interferences from the co-extracted common excipients.

Alizarin Red S (ARS) has been used as a color-developing reagent in the spectrophotometric determination of metal ions (Alkan et al., 2003; Hernandez-Mendez et al.,1983; Panahi et al., 2008; Abbaspour , Baramakeh., 2002) and pharmaceutical amines (Srikanth et al., 2010; Basavaiah et al., 1999; Kishore et al., 2010; Farhadi et al., 2003; Al Ghabsh , Al Delymi 2008; Hassan et al., 2008; Gouda et al., 2012).

The reaction between ARS and olopatadine hydrochloride has not investigated yet. Therefore, the present study was devoted to investigate the reaction between ARS and olopatadine hydrochloride, and use this color reaction in the development of simple rapid spectrophotometric method for determination of olopatadine hydrochloride in its dosage form.

2. EXPERIMENTAL

2.1 Apparatus

All of the spectrophotometric measurements were made with a Double beam UV 1800 ultraviolet visible spectrophotometer provided with matched 1-cm quartz cells (SHIMATZU- Japan)

2.2 Chemicals and solutions

All chemicals were of analytical or HPLC grade. Double distilled water was used in all experiments.
The standard of olopatadine hydrochloride was purchased by (Sigma-Aldrich, St. Louis, USA) and eye drops formulation Patanol (Kyowa Hakko Kogyo Co. Ltd. Japan) was purchased from a local pharmacy with labeled amount 0.1% solution (1mg/mL) Olopatadine hydrochloride. Alizarin Red S was supplied by (Hopkin & Williams LTD (England).

2.3 Preparation of standard solutions

2.3.1 Stock standard solution of olopatadine hydrochloride

An accurately weighed amount (0.200 g) of olopatadine hydrochloride was dissolved in double distilled water, transferred into a 100 mL standard flask, completed to the mark with the same solvent to obtain a stock solution of 2000µg/mL. The stock solution was found to be stable for at least two weeks when kept in refrigerator. The stock solution was further diluted with water to obtain working solution of 200 µg/mL.

2.3.2 Standard solution of Alizarin Red Sulphonate (ARS)

An accurately weighed amount (0.0500 g) of ARS was dissolved in double distilled water, transferred into a 100 mL standard flask, completed to the mark with the same solvent to obtain a solution of 0.05% (w/v). The solution was freshly prepared and protected from light during use.

2.3.3 Standard solution of HCl

An accurate 0.83 mL of HCl solution was transferred into a 100 mL standard flask and completed to the mark with the double distilled water to obtain standard solution of 0.1 M.

2.3.4 Pharmaceutical formulation sample

An accurate volume from sterile eye drops contains Olopatadine hydrochloride equivalent to (0.005 g) was carefully transferred into 25 mL standard flask, the volume diluted to the mark with double distilled water to obtain a stock solution of 200 µg/mL , this stock solution was diluted quantitatively with distilled water to obtain suitable concentration for the analysis by the spectrophotometric method.

2.4 General recommended procedure

Accurate volumes of olopatadine hydrochloride standard solution equivalent to (10-60 µg/mL) were transferred into a series of 100 mL separating funnels , 2 mL of 0.1 M HCl solution was added, followed by 3 mL of 0.05 % ARS. The total volume was adjusted to 10 ml by adding distilled water. The content was mixed well and after 5 min, the formed ion-pair complex was extracted with 10.0 ml of chloroform after shaking for 2.0 minutes. The two phases were allowed to separate and the chloroform layer was passed through anhydrous sodium sulphate and the absorbance was measured at λ_{max} 425 nm against blank prepared in the same manner. A calibration graph was drawn and regression equation calculated.

2.5 Determination of Stoichiometric Ratio

The Job's method of continuous variation was employed (Rose.,1964). Equimolar ($2.5x10^{-3}$) aqueous solutions of olopatadine hydrochloride and (ARS) were prepared .Series of 10 mL portions of the master solutions of Olopatadine Hydrochloride and ARS were made up comprising different complementary properties (0:10, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1, 10:0). The solution was further treated as described recommended procedure.

3. RESULTS AND DISCUSSION

3.1 Absorption spectra

As shown in Figure 1 the absorption spectrum of yellow ion-pair complex of olopatadine HCl and ARS in acidic medium which extracted into chloroform was recorded at 350-600 nm against reagent blank solution the complex shows a maximum absorbance at 425 nm , hence this wavelength was used for all subsequent measurements . Under the same conditions the reagent blank gave negligible absorbance.

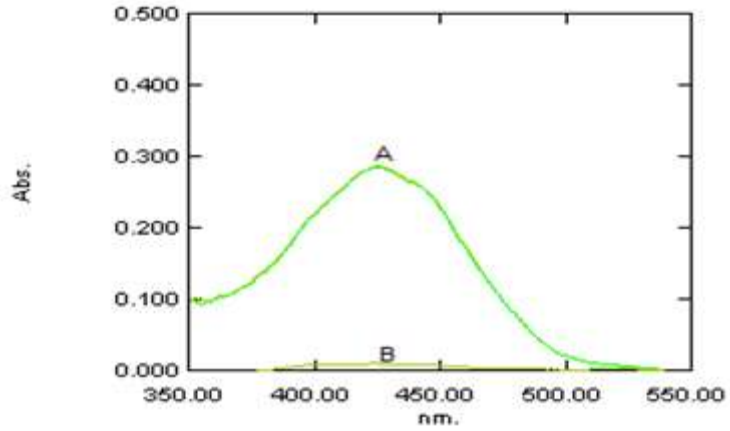

Figure 1. UV-Vis spectra of (A) yellow complex of (20 µg/mL) Olpatadine Hydrochloride and ARS (B) blank reagent.

3.2 Optimization of reaction variables

Reaction conditions were optimized by varying one parameter at a time while keeping other parameters constant and observing its effect on the absorbance at respected wavelength.

3.2.1 Effect of pH of solution on the ion-pair complex formation

The effect of pH of the aqueous phase was studied by extracting the colored complex in the presence of 5 mL acidic buffers of pH (1.0-4.0). Figure 2, revealed the decrease in the absorbance reading with the increase of pH most probably due to the interference of the H_3O^+ and ARS, and as a result diminish the complexation power. Hence, using of 0.1 M Hydrochloric acid observed maximum absorbance, no remarkable changes were observed while using different volumes of HCl of 1.0–5.0 mL, 2 mL of 0.1 M HCl selected, and it gave reproducible results.

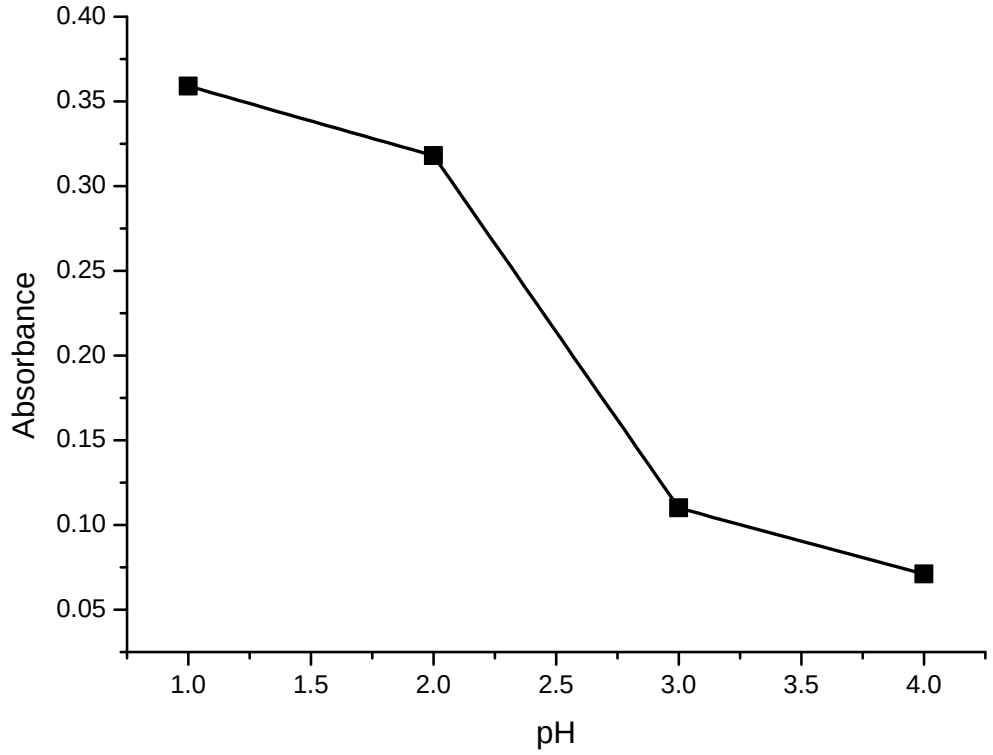

Figure 2. Effect of pH on the absorbance of (20 µg/mL) Olopatadine HCl complex with 2 mL from 0.05% ARS and 5 mL buffer solution.

3.2.2 Selection of the extracting solvent

Four immiscible organic solvents (benzene, Hexane, n-butyl alcohol and chloroform) were examined in order to provide an application procedure. The maximum absorption of the yellow ion-pair complex obtained in chloroform was selected as an extracting solvent, mainly due to its lower volatility, greater stability of extracted ion-pair complex in comparison to n- butyl alcohol.

3.2.3 Effect of reagent concentration

The effect of ARS concentration on the reaction with olopatadine HCl was investigated. Figure 3 revealed that the extraction of 20 µg/mL drug in the presence of various concentration ARS in organic phase increase with the increasing of ARS concentration in the aqueous phase. The highest absorption of ion-pair complex was attained when the volume of ARS reaches to 3.0 mL of 0.05% ARS concentration.

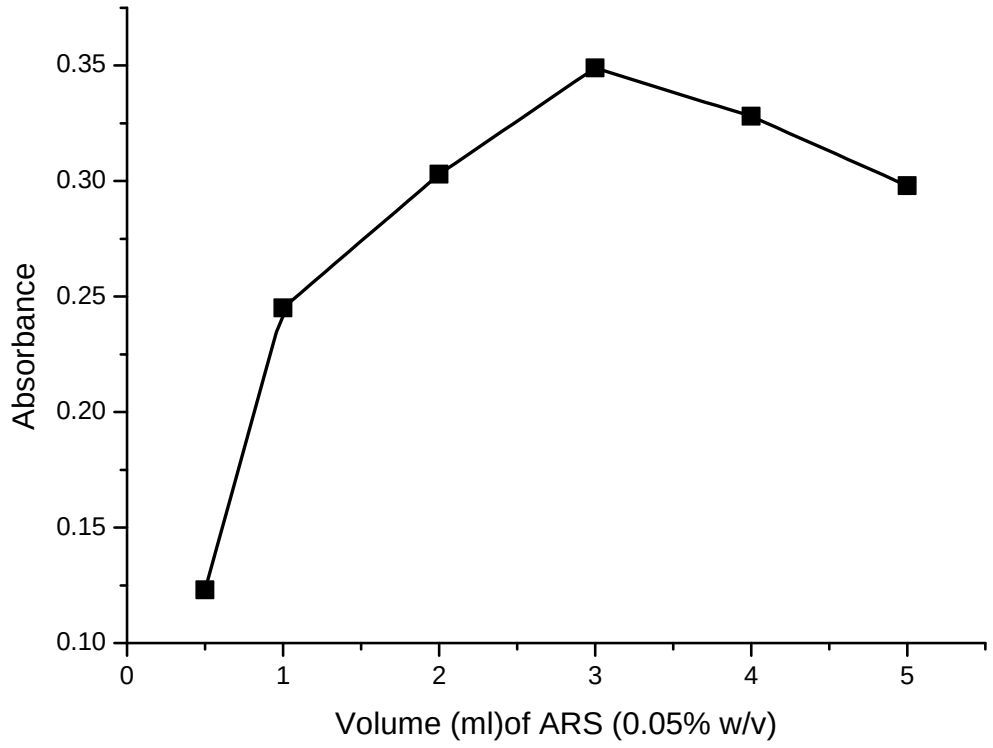

Figure 3. Effect of ARS concentration 0.05% (w/v) on the absorbance of Olopatadine HCl (20µg/mL)

3.2.4 Effect of time, sequence of additions and stability

The effect of reaction time between olopatadine HCl and ARS in acidic medium was studied by carrying out the reaction in the range of 0–25 min before extraction and it was found that 5 min was the minimum time to achieve maximum absorbance of ion-pair complex at 425 nm. Shaking time of 0.5–5 min was studied and the results showed that 2.0 min was sufficient to produce a constant absorbance. There was no appreciable change in the absorbance of the measured species if the order of reactants was varied. Complex was stable for more than 48 hours at room temperature.

3.2.5 Effect of number of extractions and volume of organic solvent

The effect of volume of aqueous Phase (included drug, ARS, HCl) was studied by using different volumes of 10, 15, 20, 25 mL of aqueous phase, and the number of extraction times was also studied. Maximum absorbance was obtained by using one extraction with 10 mL of chloroform.

3.3 Stoichiometric relationship

Under the optimum conditions the stoichiometric ratio of the ion-pair complex formed between olopatadine HCl and ARS was found to be 1:1 (Figure 4). Based on this ratio, the reaction pathway was postulated to be proceeded as shown in Figure 5. Olopatadine HCL was found to be susceptible for reaction with ARS producing yellow color product.

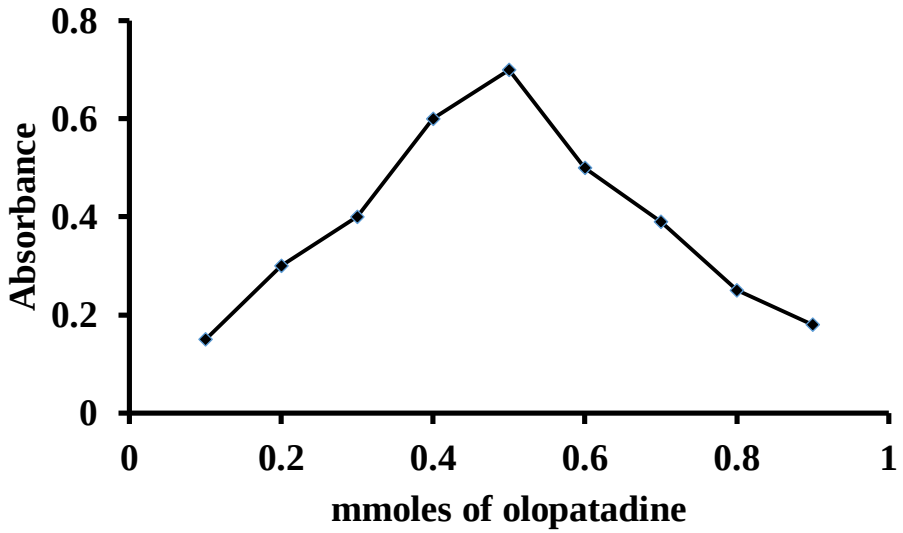

Figure.4. Job's method for Olopatadine HCl-ARS complex.

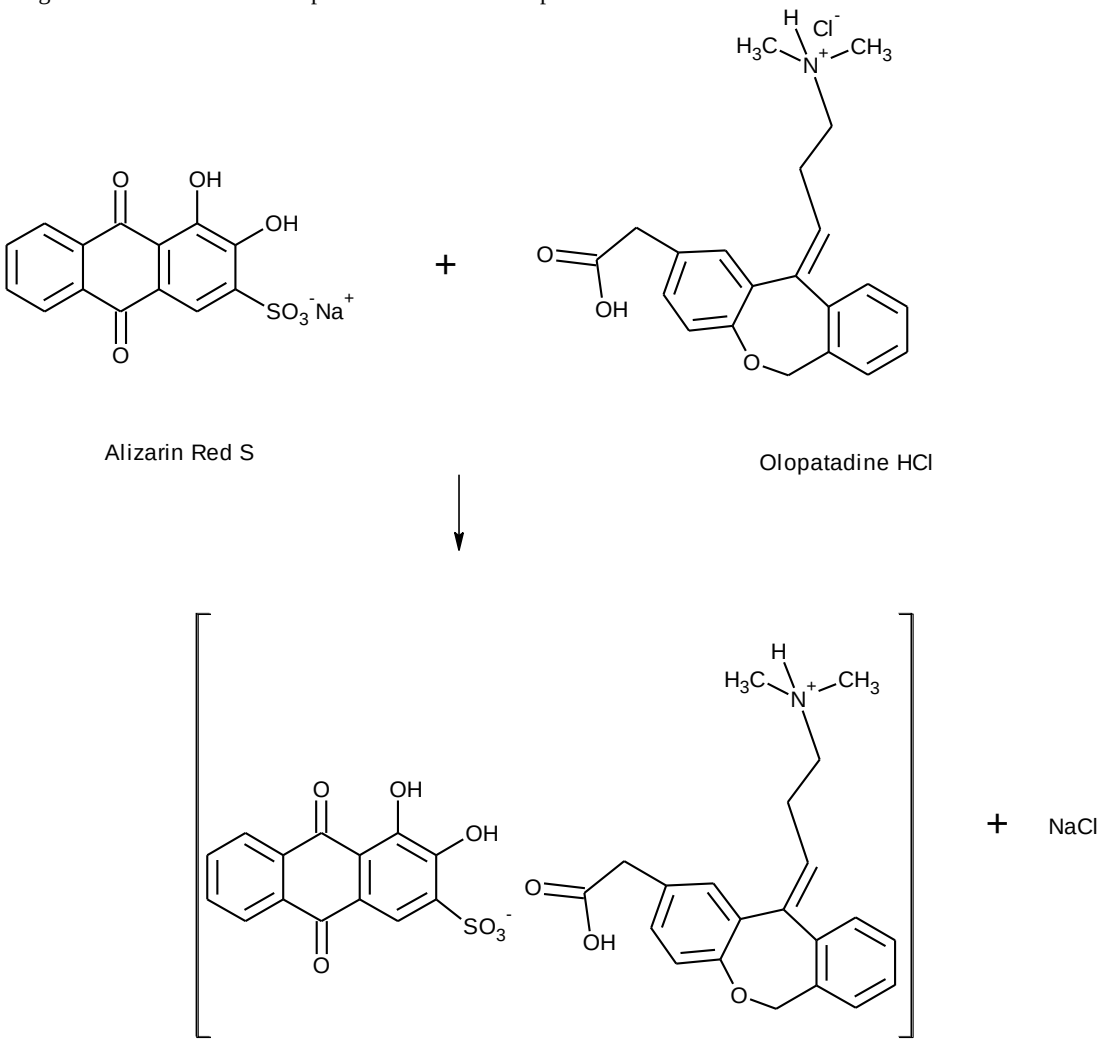

Figure 5. Scheme for the reaction pathway of Olopatadine with ARS

3.4 Validation of the method

3.4.1 Linearity

A calibration graph was constructed using a standard solution of olopatadine hydrochloride under the optimum experimental conditions; a linear relationship existed between the absorbance and concentration of drug. The linear regression equation, standard deviation, slope and intercept, correlation coefficients, and linearity ranges were given in Table1 for proposed spectrophotometric method. The molar absorptivity and Sandell sensitivity of method was calculated from Beer's law.

3.4.2 Sensitivity

The limit of detection (LOD) and limit of quantitation for the proposed method was calculated using the following equations, respectively (ICH ., 1996)

LOD = 3.3Q/S, LOQ = 10Q/S, where Q is the standard deviation of replicate determination values under the same conditions as for the sample analysis in the absence of the analyte and S is the slope of the calibration curve. According to these equations, the LOD and LOQ were calculated and listed in Table1.

Table 1. Analytical parameters of the proposed method

Parameters	Results
Linear range(µg/mL)	10 - 60
Maximum wave length(nm)	425
Regression equation (Y^*) Intercept(A) Slope(B)	 0.03667 0.01456
Correlation coefficient r	0.99956
limit of detection ,LOD(µg/mL)	2.0579
Limit of quantitation ,LOQ (µg/mL)	6.2363
Molar absorptivity ,(L mol^{-1} cm^{-1})	$7.1402x10^3$
Sandell sensitivity	0.0524

3.4.3 Specificity, precision, and accuracy

In order to determine the accuracy and precision of the method, solutions containing three different concentrations of the studied drug was prepared and analyzed. Percentage relative standard deviation (R.S.D. %) as precision indicating reasonable repeatability of the selected method and percentage relative error (Er %) as accuracy of the suggested method was calculated. Precision was carried out by three determinations at three different concentrations, Table 2.

Table.2. Evaluation of accuracy and precision

Amount taken	Amount found	Relative error (%)	Percentage ± RSD (n=3)
10	10.829	0.083	108.285± 2.320
30	29.830	-0.566	99.435 ± 0.212
50	49.496	-1.07	98.992 ± 0.202

3.4.4 Robustness

For the evaluation of the method robustness, some parameters were interchanged, HCl volume, Reagent concentration, and shaking time. The capacity remains unaffected by small deliberate variations, suggesting that the developed method was robust Table 3.

Table 3. Robustness of the proposed method

Recommended condition	recovery % ± SD (n=3)
optimum	97.183± 1.16
Volume of (0.1 M) HCl (mL) 2.3 1.7	 101.36 ± 0.80 99.99 ± 2.26
Volume of 0.05 (w/v) ARS	

3.2 2.8	100.68 ± 0.64 96.27 ± 0.34
Shaking time (min) 2.5 1.5	 103.34 ± 0.31 97.13 ± 1.83

3.4.5 Application of the proposed method

The proposed method was applied to the pharmaceutical formulation containing Olopatadine HCl. The result was shown in Table 4, indicate the high accuracy of the proposed method for the determination of the studied drug. The proposed method has the advantage of being virtually free from interferences by excipients.

Table 4. Result of analysis of dosage forms of patanol eye drop containing olopatadine hydrochloride

Formulation drug	Found % ± RSD (n=6)
Patanol 0.1% solution (1mg/mL)	97.183 ± 1.16

4. CONCLUSION

The method developed for quantitative analysis of Olopatadine HCl is rapid, precise, accurate, and selective. The method was completely validated as per ICH guidelines and satisfactory results were obtained for all the characteristics tested. The method can be conveniently used for assay of olopatadine HCl in the pharmaceutical dosage form. The method can be conveniently used in quality control laboratory.

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